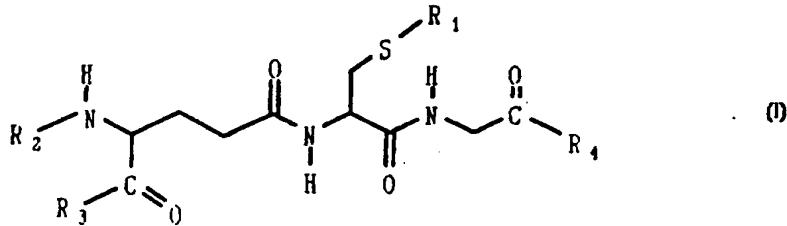




## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification <sup>6</sup> : <b>C07K 5/02, A61K 38/05</b>		A1	(11) International Publication Number: <b>WO 98/09986</b>
(21) International Application Number: <b>PCT/GB97/02358</b>		(43) International Publication Date: <b>12 March 1998 (12.03.98)</b>	(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).
(22) International Filing Date: <b>1 September 1997 (01.09.97)</b>			
(30) Priority Data: 9618277.9 2 September 1996 (02.09.96) GB 9700896.5 17 January 1997 (17.01.97) GB			
(71) Applicant (for all designated States except US): THE MANCHESTER METROPOLITAN UNIVERSITY [GB/GB]; All Saints Building, All Saints, Manchester M15 6BH (GB).		Published	With international search report.
(72) Inventor; and			
(75) Inventor/Applicant (for US only): D'SILVA, Claudio [GB/GB]; 23 Chiltern Drive, Hale/Altringham, Cheshire WA15 9PL (GB).			
(74) Agents: EVANS, David, Charles et al.; F.J. Cleveland & Company, 40-43 Chancery Lane, London WC2A 1JQ (GB).			

## (54) Title: S-BLOCKED GLUTATHIONES



## (57) Abstract

This invention concerns glutathione derivatives, and in particular their application in the suppression of pathogens. It has been discovered that certain glutathione derivatives are effective inhibitors of the growth of a range of cancer cell types, and certain micro-organisms. According to one aspect of the present invention there is provided a glutathione having structure (I). Compounds based upon this general structure are disclosed which are active against parasitic infectious agents such as *T. Brucei* and *L. Donovanii*. Further compounds are disclosed which are active against cancer cells.

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakhstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

## S-Blocked Glutathiones

This invention concerns glutathione derivatives, and  
5 in particular their application in the suppression of  
pathogens.

Illness may be caused by many agents. Bacterial  
infections are caused by micro-organisms which  
10 multiply rapidly and cause a number of diseases.  
Parasites are organisms which live in or on a host and  
feed off the host. Cancers are evident by the  
uncontrolled multiplication of cells in the body. The  
cancer may be localized, such as breast cancer, or  
15 systemic such as leukaemia.

The treatment of the illnesses caused by the  
aforementioned agents has been the subject of much  
research, and many different approaches. One approach  
20 involves targeting the cells which cause the disease  
and destroying them or disrupting their ability to  
multiply.

For such an approach to be successful the agent or  
25 drug used to attack the diseased cells should not harm  
significantly other healthy cells. Thus such an

approach requires an understanding and identification of the biochemical processes carried out in cells, and the targeting and disruption of specific processes which are unique to the diseased cells.

5

Such an approach involves the use of the cytotoxic compound methylgloxal which is produced in the cells of certain organisms. A build up of methylgloxal in the cell up to cytotoxic levels will, of course, 10 result in cell death. By inducing a build up of methylgloxal, significant growth inhibition effects have been seen in tumour cells, *Escherichia coli*, *Saccharomyces cerevisiae* and *Leishmania donovani*.

15 The build up of methylgloxal may be promoted by the inhibition of glyoxalase I (GLI) enzyme. A wide range of inhibitors of GLI are known and these include substrate or product analogues and mechanism-based inhibitors.

20

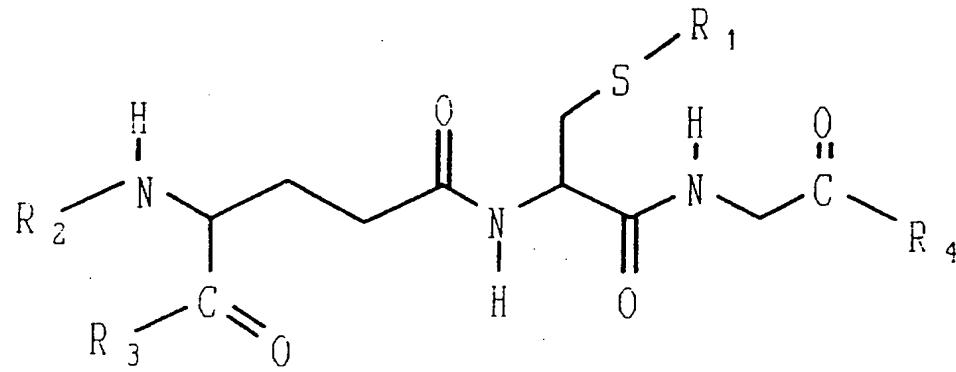
Glutathione ( $\gamma$ -glutamylcysteinylglycine) fulfils a variety of roles vital to life processes. It functions as a co-enzyme, co-substrate, substrate or part of the substrate architecture. S-blocked glutathiones have 25 been shown to be potent inhibitors of GLI in vitro and this has led to a search for particular S-blocked

glutathiones which may have therapeutic effect.

A general procedure for the preparation of the monoglycyl and dimethyl ester and amide derivatives of 5 S-(4-bromobenzyl)glutathione has been described by the inventor in Biochem. J. (1990) 271, pp167-169.

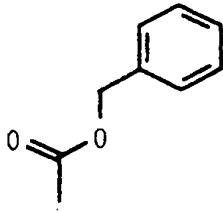
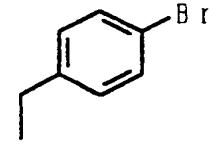
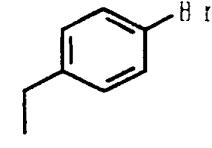
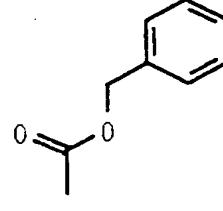
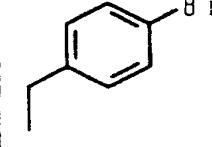
The inventor has discovered that certain glutathione derivatives are effective inhibitors of the growth of 10 a range of cancer cell types, and certain micro-organisms.

According to one aspect of the present invention there is provided a glutathione having the following 15 structure:-



4

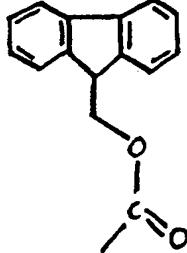
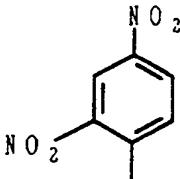
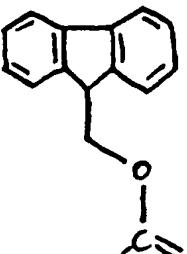
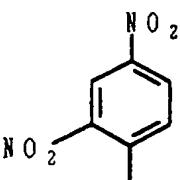
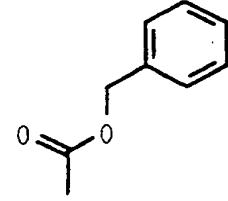
Where R1-R4 may be configured as shown in the following:

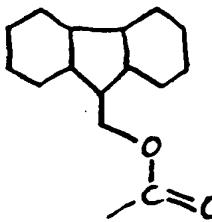
LABEL	R <sub>2</sub>	R <sub>1</sub>	R <sub>4</sub>	R <sub>3</sub>	MW
5 CD4			OMe	OMe	638. 5
CD6	CH <sub>3</sub> CO		OH	OH	545
CD7			OMe	OH	624

CD8		$\text{CH}_2\text{-COOEt}$	OH	OH	527
CD10			OMe	OMe	692
LABEL	$\text{R}_2$	$\text{R}_1$	$\text{R}_4$	$\text{R}_3$	MW
CD13	H		OH	OH	-

6

CD16	CHO		OH	OH	504
CD17			NH <sub>2</sub>	OH	576
CD19			OMe	OH	621
CD20			OMe	OMe	635

CD42			OMe	OH	708
CD43			OMe	OMe	722
CD46		$-\text{CH}_2\text{-CO}_2\text{Et}$	OMe	OMe	555

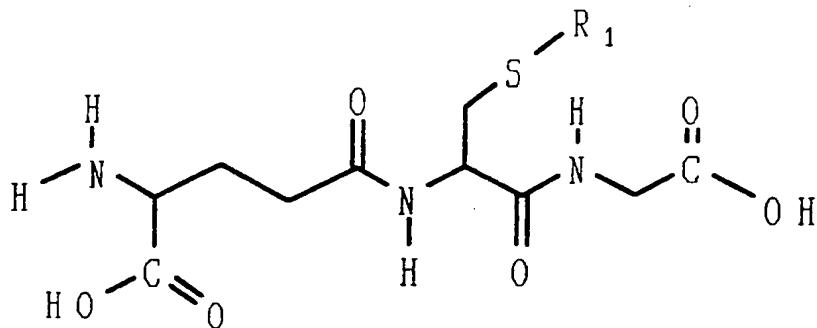
CD48	CH <sub>3</sub> CO		OMe	OH	556
------	--------------------	--	-----	----	-----

CD7 has been found to be an effective inhibitor of Trypanosomiasis and in particular T. Brucei S247. This 5 compound is also active against Malaria. One particular advantage of this compound is that it is effective at inhibiting the cell growth of the organism without being toxic to red blood cells.

10 CD13 is effective at the inhibition of growth of cancer cells, and in particular leukaemia, breast cancer or tumour cells.

According to another aspect of the invention there is 15 provided a glutathione having the following general formula:-

5 Wherein R<sub>1</sub>  
is:-



CH<sub>2</sub>CH=C(

10 CH<sub>3</sub>)CH<sub>2</sub>CH<sub>2</sub>CH=C(CH<sub>3</sub>)CH<sub>2</sub>CH<sub>2</sub>CH=C(CH<sub>3</sub>)<sub>2</sub>

TRANS TRANS TRANS

S-(farnesyl)glutathione CD37

15 The aforementioned glutathione derivatives may each be provided in pharmaceutically acceptable compositions for delivery to the human or animal body.

20 In another aspect of the invention there is provided the use of any one of the foregoing compounds in the treatment of cancer, or parasitic cellular infestation.

In particular, according to one embodiment of the present invention there is provided the use of CD13 and derivatives thereof in the treatment of cancer.

25

According to another embodiment there is provided the

10

use of CD19 and/or CD20 in the treatment of a  
infection by a parasitic micro-organism.

In particular Cd19 is highly effective at low  
5 concentrations against *T. brucei* (African sleeping  
sickness), while CD 20 has no toxicity to red blood  
cells. CD20 also has good activity against other  
parasites such as *L.donovani* (oriental sore, Kala-  
azar).

10

According to another aspect of the invention there is  
provided the use of CD7 against malaria.

According to yet another aspect of the invention there  
15 is provided the use of any one of compounds CD26-CD37  
against cancer.

According to another aspect of the invention there is  
provided the use of CD13, CD4, CD6 and CD 37 against  
20 cancer, and in particular breast cancer, and more  
particularly MCF7 cells.

Following is a description by way of example only of  
methods of putting the present invention into effect  
25 and examples demonstrating the activity of compounds  
according to the present invention. The drawing is a

11

graphic representation of the results of the in vivo test described in example 1.

General method of production.

5



Reduced glutathione (1g, 3.26mM) is dissolved in  $\text{H}_2\text{O}$  (5ml) and 2M NaOH (3.3ml, 6.6mM) with stirring at room 10 temperature and under a nitrogen atmosphere. Ethanol (5-15ml) is then added to the cloud point whereafter RX (for example, aryl halide, 3.5mM dissolved in ethanol) is added portion-wise over about 30 minutes. The reaction is left to stir for 20 hours under 15 nitrogen.

If precipitation occurs during addition either more ethanol or more water is added to dissolve the material. At the end the reaction the acidity of the 20 mixture is adjusted to p 3.5 with 2M HCl and the mixture chilled to effect precipitation. The precipitate is then filtered, washed with water, dried and recrystallized from Ethanol/ $\text{H}_2\text{O}$ .

25 Pharmaceutical activity:

Example 1

Glutathione CD13 according to the present invention was tested in order to ascertain its inhibitory characteristics with respect to various cancer cell lines.

5

The compound was introduced to cell cultures and the concentration of cancer cells formed over a period of time was measured using standard techniques. The following table indicates the results for CD13 and 10 another glutathione derivative "control" by way of comparison:-

	Leukaemia lines		Tumour cells	Breast Cancer cells
	WEH1 3B	K562	MAC 15A	MCF7
Compound	conc. $\mu$ g/ml	conc. $\mu$ g/ml	conc. $\mu$ g/ml	conc. $\mu$ g/ml
Control	36	54	>100	>100
CD13	3.4	2.5	0.43	0.48

CD13 was tested in vivo on rodents with MAC 15A S/C Tumours by giving them a 20 mg/Kg daily dose for five 20 days. The treatment resulted in a reduction in tumour size and a 45% reduction in tumour volume after 4 days. The effect of CD 13 in the above test is shown

13

in the graph of the drawings.

Example 2

5 The glutathione derivatives CD7 and CD 10 according to the present invention were tested for their activity against the parasites T.Brucei S247, L.donovani and T. cruzi.

10 The following table shows the mean estimation of growth of T.Brucei S247 in a 72 hour incubation in the presence of a control glutathione derivative and CD10 in various concentrations:-

15	Compound	MIC @ concentration ( $\mu$ M)			
		30	10	3	1
	control	++++	++++	+++++	+++++
	CD7	0	++++	+++++	+++++
	CD10	0	++++	+++++	+++++

20

The forgoing table shows that Compounds CD7 and CD10 exhibit complete inhibition of the growth of T.Brucei S247 over the specified period at concentrations of 30  $\mu$ M.

25

The following table shows the results of the inhibition of the growth of *L. donovani* and *T. Cruzi* by compounds CD7, CD10 according to the present invention and "control" by way of comparison, at 5 various concentrations.

10

Compound	% Inhibition of <u>L. donovani</u> @ conc.			% Inhibition of <u>T. Cruzi</u> @ conc.		
	90 ( $\mu$ M)	30 ( $\mu$ M)	10 ( $\mu$ M)	90 ( $\mu$ M)	30 ( $\mu$ M)	10 ( $\mu$ M)
control	0	0	0	0	0	0
CD7	0	0	0	0	0	0
CD10	T	T	0	T	T	T/0

15

The foregoing shows that compound CD10 shows good activity in the inhibition of growth of both *L. donovani* and *T. cruzi*.

20

Activity of compounds CD16-CD20 in Vitro.

Compound	% inhibition @ concn. ( $\mu$ M)

	30	10	3	1
CD16				
T.cruzi	11.0	0	0	0
CD17				
T.cruzi	7.0	4.0	0	0
CD19				
T.brucei	100	100	33.2	0
CD20				
L.donovani	13.5	1.9	0	0
T.brucei	100	64.5	0	0

10

Example 4:

15 Compound CD48 was tested for its activity against cancer, with the following results for a range of cancer types:

Cancer	Human Ovarian carcinoma	Human lung carcinoma	Human colon carcinoma	Human myclogenious leukaemia	Mouse lymphoid neoplasm
Designation	A2780	H-460	BE	K562	P388

	μM	μM	μM	μM	μM
CD48	>50	>50	27.1	16.7	31.7

Example 5:

5

Compounds CD4, CD6, and CD 37 have also been found to be effective against various cancers, including breast cancer.

10 Example 6:

Compounds CD42 to 48 were tested for their activity against various parasitic infection agents and the results are shown in the following table:

15

Comp ound	% INHIBITION OF T.BRUCEI S247				%INHIBITION OF L. DONOVANI			
	30μM	10μM	3μM	1μM	30μM	10μM	3μM	1μM
CD42	100	100	0	0	1.2	0	0	0
CD43	100	100	0	0	T/O	0	0	0
CD44	100	100	100	63.2	T/ 100	0	0	0

20

17

CD46	0	0	0	0	66.2	0	0	0
					3			

Key: T/0 = toxic to macrophages / parasites present.

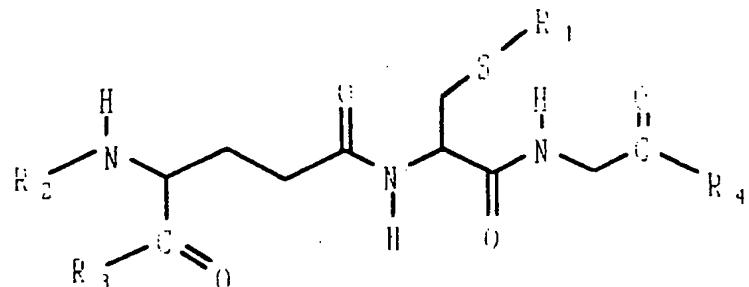
T/100 = toxic to macrophages / no parasites  
5 present.

100+ = 100% inhibition when sampling for  
haemocytometer count but parasites visible in  
wells of 96-well plate under inverted mic.

10

## CLAIMS

1. A compound having the following general structure:-

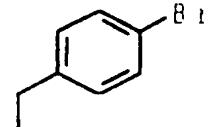
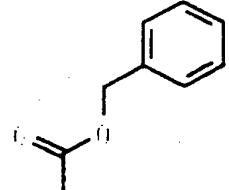
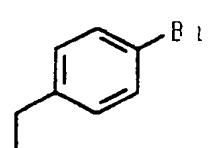
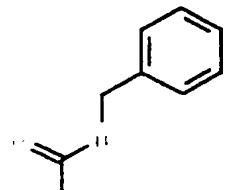


5

and wherein R<sub>1</sub> to R<sub>4</sub> are according to any one of the following rows in the table:

10

LABEL	R <sub>2</sub>	R <sub>1</sub>	R <sub>4</sub>	R <sub>3</sub>
CD4			OMe	OMe

CD6	CH <sub>3</sub> CO		OH	OH
CD7			OMe	OH
CD8		CH <sub>2</sub> -COOEt	OH	OH

CD10			OMe	OMe
CD13	H		OH	OH
CD16	CHO		OH	OH
CD17			NH2	OH

CD19			OMe	OH
CD20			OMe	OMe
CD37	H	$  \begin{aligned}  &\sim \text{CH}_2\text{CH}=\text{C}(\text{CH}_3) \\  &)\text{CH}_2\text{CH}_2\text{CH}=\text{C}(\text{CH}_3)\text{CH}_2\text{CH}_2\text{CH} \\  &\quad =\text{C}(\text{CH}_3)_2  \end{aligned}  $	OH	OH

CD42			OMe	OH
CD43			OMe	OMe
CD46		-CH <sub>2</sub> -CO <sub>2</sub> Et	OMe	OMe

CD48	CH <sub>3</sub> CO		OMe	OH
------	--------------------	--	-----	----

2. A pharmaceutically acceptable composition for  
5 delivery to the human or animal body comprising a  
compound according to claim 1.

10 3. A composition for the treatment of parasitic  
infection, which composition comprises one or  
more of CD7, CD19, CD20, CD42, CD43 or CD44  
according to claim 1.

15 4. A composition for the treatment of  
trypanosomiasis, and in particular infection by  
T. Brucei, which composition comprises one or  
more of CD7, CD19, CD20, CD42, CD43 and CD44  
according to claim 1.

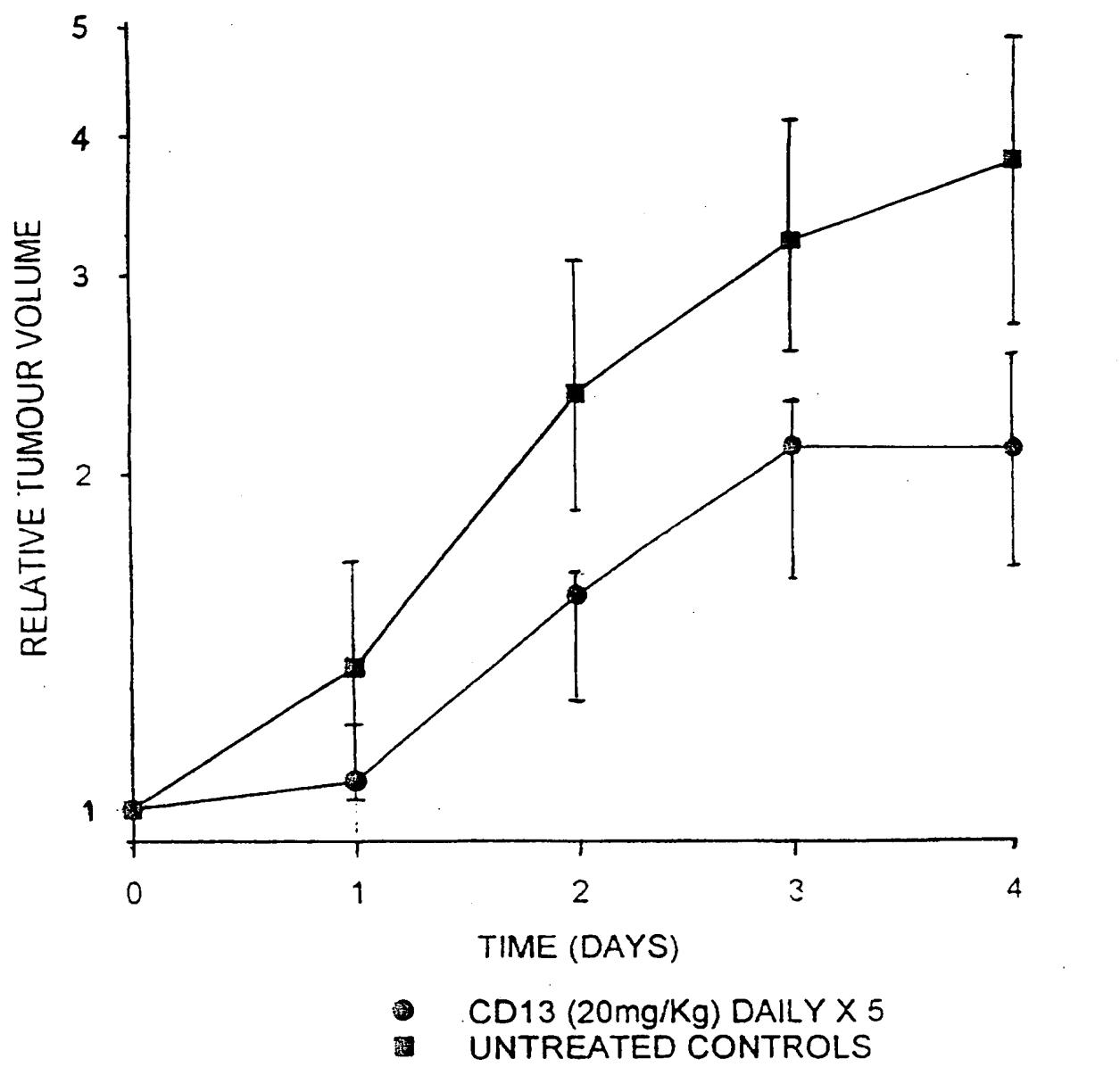
20 5. A composition as for the treatment of  
Leishmaniasis, and in particular infection by L.  
donovani, which composition comprises one or  
more of CD20, CD42, CD43, CD44 and CD46 according

to claim 1.

6. A composition for the treatment of cancer, which composition comprises one or more of CD4, CD6, 5 CD13 and CD37 according to claim 1.
7. A method of treating a diseased human or animal comprising administering a pharmologically effective amount of a composition as claimed in 10 claim 2.
8. A method of treating parasitic infection of a human or animal comprising administering a pharmologically effective amount of a composition as claimed in any of claims 3,4 and 5. 15
9. A method of treating cancer in a human or animal comprising administering a pharmologically effective amount of a composition as claimed in 20 claim 6.

1/1

## MAC15A S/C TUMOURS TREATED WITH CD13 DAILY



## INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 97/02358

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C07K5/02 A61K38/05

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	R.VINC ET AL.: "Studies on the Inhibition of Glyoxalase I by S-Substituted Gluthationes" J.MED.CHEM., vol. 14, no. 5, 1971, page 402-4 XP002044716 see paragraph 1; example 39; table 1 ---	1,6-9
X	A.AL-TIMARI ET AL.: "Inhibition of mammalian glyoxalase I (lactoylglutathione lyase) by N-acylated S-blocked glutathione derivatives as a probe for role of the N-site of glutathione in glyoxalase I mechanism" BIOCHIM.BIOPHYS.ACTA, vol. 86, no. 1, 1986, pages 160-8, XP002044717 see tables 12,5 ---	1,6-9



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

## \* Special categories of cited documents :

- \*A\* document defining the general state of the art which is not considered to be of particular relevance
- \*E\* earlier document but published on or after the international filing date
- \*L\* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- \*O\* document referring to an oral disclosure, use, exhibition or other means
- \*P\* document published prior to the international filing date but later than the priority date claimed

\*T\* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

\*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

\*Y\* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

\*&\* document member of the same patent family

1

Date of the actual completion of the international search

27 October 1997

Date of mailing of the international search report

13. 11. 97

## Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,  
Fax: (+31-70) 340-3016

## Authorized officer

Deffner, C-A

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 97/02358

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	A.AL-TIMARI ET AL.: "Inhibition of glutathione derivatives of bovine liver glyoxalase II (Hydroxyacetylglutathione hydrolase) as a probe of the N- and S-sites for substrate binding" BIOCHIM.BIOPHYS.ACTA, vol. 870, no. 2, 1986, page 219-25 XP002044718 see example 2; table I ---	1,6-9
A	S.J.NORTON ET AL.: "Inhibitors and inhibition of mammalian glyoxalase II activity" BIOCHEM.SOC.TRNS., vol. 21, no. 2, 1993, pages 545-9, XP002044719 ---	
A	NORTON S.J. ET AL.: "Glyoxalase and Glyoxalase II from Aloe Vera: Purification, Characterization and Comparision with Animal Glyoxalases" BIOCHEM.INT., vol. 22, no. 3, 1990, pages 411-18, XP002044720 ---	
A	AC. ELIA ET AL.: "N,S-Bis-Fluorenylmethoxycarconylglutathione: A New, Very Potent Inhibitor of Mammalian Glyoxalase II" BIOCHIM.MOL.BIOL.INT., vol. 35, no. 4, 1995, pages 763-71, XP002044721 ---	
A	WO 95 08563 A (TERRAPIN TECH INC) 30 March 1995 -----	

**INTERNATIONAL SEARCH REPORT**

Information on patent family members

Intern. Appl. No.

PCT/GB 97/02358

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9508563 A	30-03-95	US 5599903 A AU 7842194 A CA 2171453 A EP 0720620 A JP 9506336 T US 5556942 A	04-02-97 10-04-95 30-03-95 10-07-96 24-06-97 17-09-96